

RESEARCH ARTICLE

District-wide One Health surveillance of antimicrobial resistant *Escherichia coli* in surface water and groundwater in Sri LankaSavithri Jinadasa¹ , Nadisha Badanasinghe² and Sujatha Pathirage³

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ABSTRACT

Background and Aim: Environmental antimicrobial resistance (AMR) is an emerging One Health concern, yet district level evidence integrating surface water and groundwater remains limited in Sri Lanka. This study aimed to determine *Escherichia coli* contamination, characterize antimicrobial susceptibility, and compare AMR profiles between surface water and groundwater sources in Gampaha District, Sri Lanka.

Materials and Methods: A descriptive cross-sectional study was conducted from November 2023 to November 2024. A total of 104 surface water samples were collected from 52 major water bodies, while 161 groundwater samples were obtained through routine surveillance. *E. coli* was isolated by membrane-filtration and confirmed using biochemical tests. Antimicrobial susceptibility was evaluated by Kirby–Bauer disk diffusion against 10 antibiotics according to Clinical and Laboratory Standards Institute guidelines. Extended-spectrum beta-lactamase (ESBL) and AmpC production were assessed phenotypically. Multidrug resistance (MDR) was defined as resistance to ≥ 1 agent in ≥ 3 antimicrobial classes.

Results: All 52 surface water sites were contaminated with *E. coli*, and 68.3% (71/104) of samples contained ≥ 200 colony-forming units/100 mL. Among groundwater samples, 52.2% (84/161) were *E. coli*-positive. Ninety-three surface water and 84 groundwater isolates were analyzed for AMR. Nonsusceptibility to ≥ 1 antibiotic occurred in 64.5% and 59.5% of surface water and groundwater isolates, respectively. MDR prevalence was comparable between sources (19.4% vs. 19.0%). Ampicillin showed the highest nonsusceptibility (54.8% vs. 45.2%). ESBL-producing isolates occurred in 5.4% and 1.2%, whereas presumptive AmpC producers occurred in 0% and 2.4% of surface water and groundwater isolates, respectively. All isolates were susceptible to ertapenem. Resistance-category distributions did not differ significantly between water sources ($\chi^2 = 2.277$, $df = 3$, $p = 0.517$).

Conclusion: Widespread fecal contamination and clinically relevant AMR occur across surface water and groundwater in Gampaha District despite substantial differences in contamination intensity. Comparable MDR burdens across both sources highlight environmental dissemination of resistant *E. coli* and support integrated One Health surveillance. The findings provide a district level environmental AMR baseline for targeted interventions and future molecular and quantitative microbial risk assessments.

Keywords: antimicrobial resistance, environmental surveillance, *Escherichia coli*, extended-spectrum beta-lactamase, groundwater, multidrug resistance, One Health, surface water.

INTRODUCTION

Antimicrobial resistance (AMR) has emerged as one of the foremost global public health threats, with an estimated 1.27 million deaths directly attributable to bacterial AMR in 2019 [1]. Although AMR has historically been considered primarily a clinical problem, its emergence and dissemination are increasingly recognized as environmental challenges that require coordinated action within the One Health framework, which integrates human, animal, and ecosystem health [2]. Aquatic environments can serve as reservoirs and dissemination pathways for antibiotic-resistant bacteria and antibiotic resistance genes (ARGs). Fecal contamination can introduce resistant bacteria and ARGs into water systems, facilitating their persistence and potential dissemination across interconnected environmental compartments [2–10].

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In Sri Lanka, an estimated 1750-3460 deaths were directly attributable to AMR in 2021, while 6610-13,500 deaths were associated with resistant organisms [11]. National surveillance data indicate substantial antibiotic consumption, with penicillins accounting for approximately 60% of antibiotic use [12]. Such antimicrobial use may contribute to selection pressure favoring resistant bacterial populations, including resistance to commonly used penicillins such as ampicillin and amoxicillin. These concerns are compounded by high population density, rapid urbanization, inadequate sewage treatment, and challenges in regulating antibiotic use across the human health, animal health, agricultural, and aquaculture sectors [12–14]. Together, these factors underscore the importance of environmental surveillance as a component of national AMR monitoring and control.

Gampaha District, with a population of approximately 2 million and an area of 1,387 km², is the most populous and a highly industrialized district in Sri Lanka. The district contains the Biyagama Export Processing Zone, multiple hospitals and pharmaceutical outlets, and extensive paddy and aquaculture farming. These characteristics create overlapping human, agricultural, and industrial pressures within a shared hydrological network and make the district particularly relevant for environmental AMR surveillance [15–19]. Previous studies have documented antibiotic residues and resistant bacteria in the Kelani River [20, 21]. Kumara *et al.* [22] reported widespread antibiotic resistance among *Escherichia coli* isolates from inland water bodies in Sri Lanka, highlighting aquatic environments as potential reservoirs for ARG dissemination. In addition, an urban area of Gampaha District had a significantly higher prevalence of antimicrobial-resistant *E. coli* among humans, livestock, wild animals, and environmental samples than rural settings in Sri Lanka [23]. Collectively, these observations indicate that densely populated and highly anthropized environments may represent important interfaces for the environmental persistence and dissemination of AMR.

Despite this evidence, important knowledge gaps remain in environmental AMR surveillance in Sri Lanka. Previous investigations have largely focused on individual rivers, specific categories of water bodies, selected geographic locations, or broader national assessments, limiting direct comparison of AMR patterns across different water-source types within the same administrative and hydrological setting. In particular, no systematic district-wide surveillance has simultaneously evaluated surface water and groundwater. Consequently, the extent to which *E. coli* contamination and AMR profiles differ or converge between these two environmentally and epidemiologically important water sources remains unclear. Furthermore, district level data integrating microbiological contamination, antimicrobial susceptibility profiles, spatial information, and documented human exposure to surface water are limited. Addressing these gaps is important for establishing a locally relevant environmental AMR baseline and identifying whether surveillance and mitigation strategies should consider surface water and groundwater as distinct or interconnected components of the environmental AMR reservoir.

Therefore, this study aimed to determine the prevalence of *E. coli* contamination and characterize AMR in surface water and groundwater sources across Gampaha District, Sri Lanka. Specifically, the objectives were to determine the prevalence and magnitude of *E. coli* contamination in surface water and groundwater; characterize the antimicrobial susceptibility profiles of *E. coli* isolates recovered from both sources; and compare AMR patterns between surface water and groundwater. In addition, the study integrated GPS-based spatial mapping and standardized documentation of human exposure activities across 52 surface water bodies to provide a district level environmental AMR baseline. The findings were interpreted within the One Health framework and in relation to Sri Lanka's National Strategic Plan for Combating AMR 2023-2028 to support locally targeted surveillance and intervention priorities.

MATERIALS AND METHODS

Ethical approval

This study involved environmental water sampling exclusively and did not involve human participants, animals, human- or animal-derived biological specimens, or personally identifiable information. In accordance with the ethical framework applicable in Sri Lanka and the guidelines of the Ethics Review Committee, Faculty of Medicine, University of Kelaniya, formal ethical approval was not required because the study was restricted to environmental sampling and routine water quality surveillance data, with no direct human or animal participation. The authors confirm that all methods were carried out in accordance with relevant guidelines and regulations.

Study period and location

The study was conducted from November 2023 to November 2024 in Gampaha District, Sri Lanka. Surface water sampling was conducted between November 2023 and February 2024, spanning the latter part of the

second inter-monsoon/Maha season and the transition to the primary dry period. Groundwater samples were collected continuously from November 2023 to November 2024 through the routine water quality surveillance program of the National Reference Laboratory of Food and Water Microbiology, Medical Research Institute (MRI), Colombo, covering both wet and dry periods. Laboratory processing was performed at the Department of Medical Microbiology, University of Kelaniya, and the National Reference Laboratory of Food and Water Microbiology, MRI, Colombo.

Study design

A descriptive cross-sectional study was conducted to characterize *E. coli* contamination and AMR in surface water and groundwater sources across Gampaha District. The study combined systematic sampling of 52 major surface water bodies with groundwater samples obtained through the routine district-wide surveillance program at the MRI. This design enabled simultaneous characterization and comparison of *E. coli* contamination and antimicrobial susceptibility profiles between surface water and groundwater within the same administrative district. The overall study workflow is summarized in Figure 1.

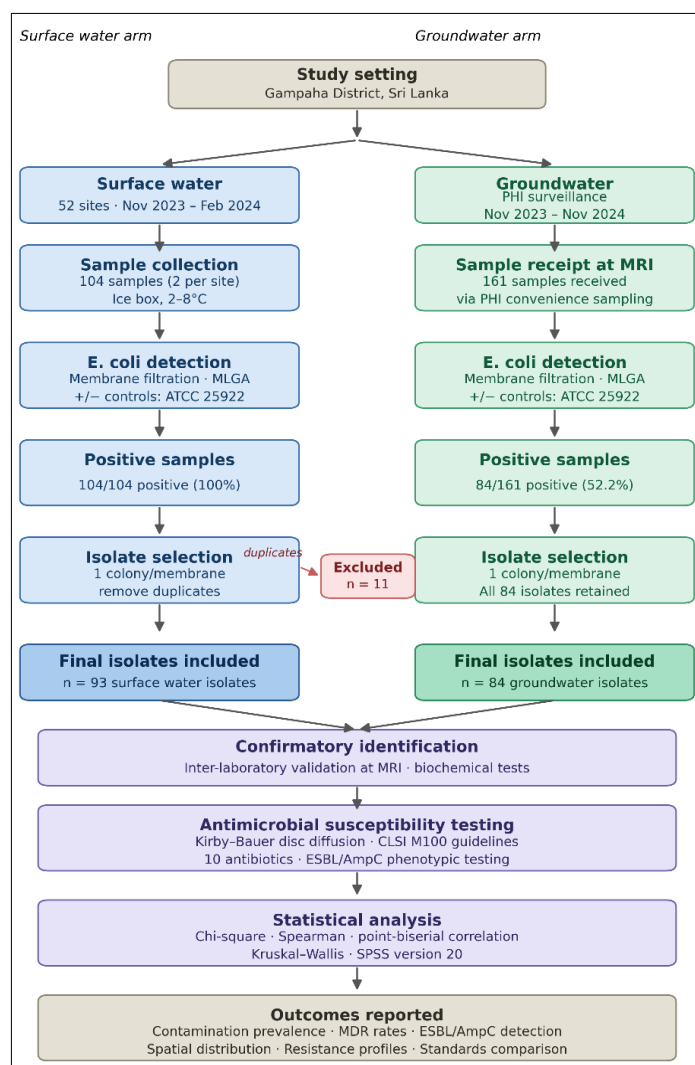


Figure 1: Flow diagram of the study methodology. PHI = Public Health Inspector; MRI = Medical Research Institute; MLGA = Membrane Lactose Glucuronide agar; AST = Antimicrobial susceptibility testing; CLSI = Clinical and Laboratory Standards Institute; MDR = Multidrug resistance; ESBL = Extended-spectrum beta-lactamase.

Surface water sampling

The minimum required sample size for surface water was calculated using the formula:

$$n = Z^2 p(1 - p)/d^2,$$

where $Z = 1.96$ for a 95% confidence level, $p = 0.75$ based on the expected prevalence of *E. coli* contamination reported by Mahagama and Manage [24] for natural water sources in Anuradhapura District, Sri Lanka, and $d = 0.10$ as the acceptable margin of error. The calculated minimum sample size was 72 [25].

A total of 52 major surface water sites in Gampaha District were identified by cross-referencing major agricultural reservoirs listed in the 2012 Census of Agriculture with additional major water bodies identified using Google Maps. Two sampling points approximately 500 m apart were selected at each site from locations where human contact with water was observed, yielding 104 surface water samples from rivers, streams, canals, tanks, lakes, and ponds. GPS coordinates were recorded for each sampling site using a handheld GPS device with an accuracy of ± 5 m, and human exposure activities were documented using a standardized field checklist. The locations of the 52 surface water sampling sites are presented in Figure 2. Samples were collected aseptically in sterile glass bottles, transported on ice at 2–8°C with temperature monitoring, delivered to the laboratory within 6 h, and processed within 24 h.

Surface water sampling was conducted as a single round between November 2023 and February 2024, covering the latter part of the second inter-monsoon/Maha season and transition to the primary dry period. Sampling under relatively stable, low-flow conditions was selected because monsoonal flooding can increase hydrological connectivity and cross-contamination among surface water bodies in low-lying areas of Gampaha District, potentially limiting source-specific comparisons. This sampling strategy enabled standardized collection across the 52 sites under broadly comparable hydrological conditions. However, because peak Southwest monsoon conditions were not sampled, the study did not capture potential increases in runoff and mobilization of fecal contamination, antibiotic residues, and antimicrobial-resistant bacteria during periods of intense rainfall. This seasonal constraint is considered in the study limitations.

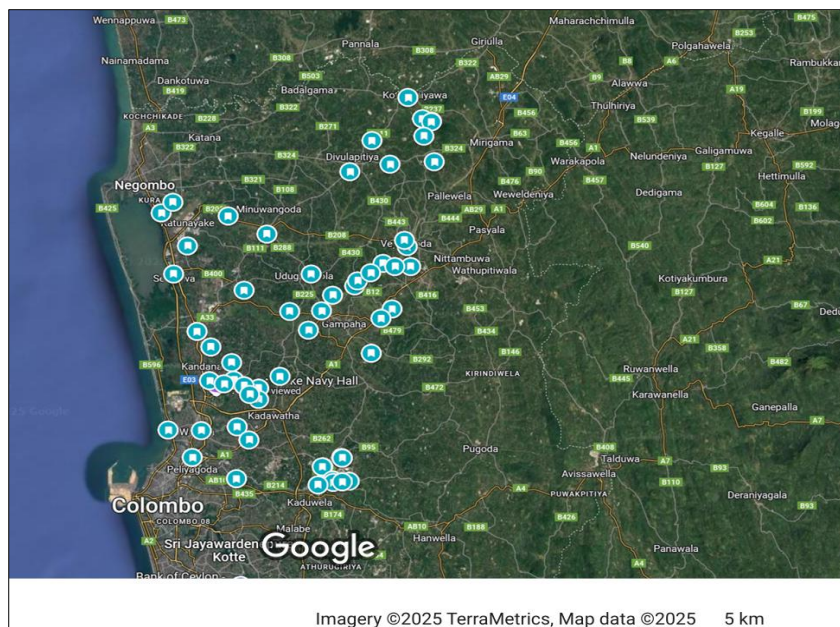


Figure 2: GPS-mapped locations of surface water sampling sites in Gampaha District, Sri Lanka.

Groundwater sampling

Groundwater samples ($n = 161$) were obtained from dug and tube wells used for drinking water and enrolled in the routine water quality surveillance program of the National Reference Laboratory of Food and Water Microbiology, MRI, Colombo. Samples were collected by Public Health Inspectors (PHIs) throughout Gampaha District and submitted to the MRI as part of scheduled surveillance activities. All groundwater samples received from Gampaha District between November 2023 and November 2024 were included, with no additional exclusion criteria applied by the research team. Samples were received throughout the study period, including wet and dry periods. Information regarding the number of dug versus tube wells and well depth was unavailable in the routine surveillance dataset. All *E. coli* isolates recovered from groundwater samples ($n = 84$) were subsequently processed for confirmatory identification and antimicrobial susceptibility testing (AST).

Selection of wells for surveillance sampling was determined by the respective PHIs and was not randomized or stratified according to geographic zone, well type, or depth. This approach was used because a comprehensive and current sampling frame of drinking water wells in Gampaha District was unavailable, and the MRI surveillance program provided the practical mechanism for district-wide groundwater sampling. Consequently, wells suspected of contamination or serving vulnerable populations may have been preferentially submitted, whereas wells in remote or underserved areas may have been underrepresented. Therefore, groundwater prevalence

estimates represent the monitored well population and may not be generalizable to the entire groundwater supply of Gampaha District.

Microbiological processing

Water samples were processed using the standard membrane-filtration method with 0.45- μ m-pore-size nitrocellulose membranes in accordance with ISO 9308-1:2014 [26]. A 100-mL aliquot of each water sample was filtered, and the membrane was incubated on Membrane Lactose Glucuronide agar (MLGA) (Oxoid, Basingstoke, United Kingdom) to differentiate *E. coli* from other coliforms. Each membrane-filtration batch included positive and negative controls using *E. coli* American Type Culture Collection (ATCC) 25922 and sterile distilled water, respectively.

Presumptive *E. coli* colonies were confirmed using Gram staining and catalase, oxidase, motility, indole, ortho-nitrophenyl- β -galactoside, urease, citrate, and methyl red-Voges-Proskauer tests. A single representative colony per membrane was selected for AST to minimize inclusion of clonal replicates originating from the same water sample. All confirmed groundwater *E. coli* isolates ($n = 84$) recovered at the MRI were transported to the University of Kelaniya for independent confirmatory identification and AST; no discrepant results were observed.

Media performance was verified for each batch by confirming the characteristic green colonies of *E. coli* ATCC 25922 as the positive control and absence of growth of *Bacillus subtilis* ATCC 6633 as the negative control. Molecular confirmation was not performed. Identification of *E. coli* was therefore based on MLGA chromogenic culture combined with eight confirmatory biochemical tests in accordance with the laboratory approach based on ISO 9308-1:2014 [26].

AST

AST was performed using the Kirby–Bauer disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) M100 Performance Standards for Antimicrobial Susceptibility Testing, 34th edition [27]. Ten antimicrobial agents were tested: ampicillin (AMP; 10 μ g), amoxicillin-clavulanate (AMC; 20/10 μ g), cefotaxime (CTX; 30 μ g), ceftazidime (CAZ; 30 μ g), ciprofloxacin (CIP; 5 μ g), trimethoprim-sulfamethoxazole (SXT; 1.25/23.75 μ g), gentamicin (CN; 10 μ g), tetracycline (Tet; 30 μ g), chloramphenicol (C; 30 μ g), and ertapenem (ETP; 10 μ g).

Mueller-Hinton agar (Oxoid, Basingstoke, United Kingdom) was quality controlled for pH (7.2–7.4), depth (4 mm), and sterility according to CLSI recommendations. Antibiotic disk quality control was performed using *E. coli* ATCC 25922 and *E. coli* ATCC 35218 for amoxicillin-clavulanate, and inhibition-zone diameters were verified against the quality control ranges specified in CLSI M100, 34th edition [27]. When isolates obtained from the same surface water site exhibited identical susceptibility profiles against all 10 antimicrobial agents, only one representative isolate was retained for analysis.

Detection of beta-lactamase production

Extended-spectrum beta-lactamase (ESBL) production was confirmed using the combination disk test with cefotaxime and ceftazidime tested alone and in combination with clavulanate according to CLSI M100, 34th edition [27]. Phenotypically presumptive AmpC beta-lactamase production was assessed based on the cefoxitin inhibition-zone diameter in combination with resistance to ceftazidime and cefotaxime [28]. Quality control for ESBL confirmation was performed using *Klebsiella pneumoniae* ATCC 700603 and *E. coli* ATCC 25922 as positive and negative controls, respectively, according to CLSI guidelines.

Statistical analysis

Isolates were categorized as susceptible, intermediate, or resistant according to CLSI breakpoints. For the analysis and surveillance reporting, isolates classified as intermediate or resistant were combined into a single “nonsusceptible” category. This approach is consistent with the use of nonsusceptibility categories in antimicrobial resistance surveillance, as described in CLSI M39. Multidrug resistance (MDR) was defined as resistance to ≥ 1 antimicrobial agent in ≥ 3 antimicrobial classes [29]. Proportions were reported with 95% confidence intervals (CIs) calculated using the Wilson score method. Fisher's exact test (two-tailed; $\alpha = 0.05$) was used to compare individual AMR rates between groups. The Chi-square test was used to compare resistance-category distributions between source types and MDR proportions among water body types. The Kruskal-Wallis test was used to compare the number of resistant antimicrobial classes among rivers/streams/canals, tanks, lakes, and ponds.

Binary logistic regression was performed to assess independent predictors of MDR, with water body type and log-transformed *E. coli* count included as covariates. Spearman's rank correlation coefficient (ρ) was used to assess the relationship between colony count and the number of resistant antimicrobial classes, whereas the point-biserial correlation coefficient (r) was used to evaluate the association between colony count and binary MDR status. No correction for multiple comparisons was applied across the 10 antimicrobial agents. With 10 simultaneous comparisons at $\alpha = 0.05$, the probability of ≥ 1 false-positive result was approximately 40%; therefore, borderline p-values were considered exploratory. All statistical analyses were performed using IBM SPSS Statistics version 20.0 (IBM Corp., Armonk, NY, USA).

RESULTS

Surface water contamination

A total of 104 surface water samples were collected from 52 major surface water bodies in Gampaha District, with two sampling points located approximately 500 m apart at each site (Table 1).

Table 1: Surface water sampling sites according to water body type in Gampaha District (n = 52 sites)

Water body type	Sites, n	Percentage (95% CI)
Rivers/streams/canals	22	42.3% (29.6-56.1%)
Tanks	9	17.3% (9.4-29.4%)
Lakes	11	21.2% (12.3-34.0%)
Ponds	10	19.2% (10.9-31.7%)

Percentages may not sum to 100% due to rounding. CI = Confidence interval.

Human exposure to the sampled surface water sources was common. Fishing was observed at 49/52 (94%) sites, agricultural use at 32/52 (62%), bathing/clothes washing at 31/52 (60%), lotus picking at 19/52 (37%), and drinking water use at 4/52 (8%) sites (Table 2).

Table 2: Human exposure activities observed at surface water sampling sites in Gampaha District (n = 52 sites)

Exposure activity	Sites, n	Percentage (95% CI)
Drinking	4	8% (3.2-18.9%)
Bathing/clothes washing ¹	31	60% (46.4-72.2%)
Fishing	49	94% (83.8-98.0%)
Agriculture	32	62% (48.2-73.9%)
Lotus picking	19	37% (25.2-50.7%)

¹Clothes washing was observed at the same 31 sites as bathing and was therefore included in the same category. CI = Confidence interval.

All 52 surface water sites were positive for *E. coli*. Total coliform counts exceeded 200 colony-forming units (CFU)/100 mL in all samples. *E. coli* counts exceeded 200 CFU/100 mL in 68.3% (71/104; 95% CI: 58.8-76.4%) of samples, were 100-199 CFU/100 mL in 14.4% (15/104; 95% CI: 8.9-22.4%), and were 0-99 CFU/100 mL in 17.3% (18/104; 95% CI: 11.2-25.7%) (Table 3).

Groundwater contamination

Among the 161 groundwater samples received through routine surveillance from Gampaha District, 52.2% (84/161; 95% CI: 44.5-59.7%) were positive for *E. coli*. Among the 84 *E. coli*-positive groundwater samples, counts were 0-99 CFU/100 mL in 88.1% (74/84; 95% CI: 79.5-93.4%), 100-199 CFU/100 mL in 4.8% (4/84; 95% CI: 1.9-11.6%), and ≥ 200 CFU/100 mL in 7.1% (6/84; 95% CI: 3.3-14.7%) (Table 3).

Table 3: Comparison of *Escherichia coli* colony counts in surface water and *E. coli*-positive groundwater samples in Gampaha District

<i>E. coli</i> colony-count (CFU/100 mL)	Surface water (n = 104), n (%)	Groundwater (n = 84), n (%)
0-99	18 (17.3%; 95% CI: 11.2-25.7%)	74 (88.1%; 95% CI: 79.5-93.4%)
100-199	15 (14.4%; 95% CI: 8.9-22.4%)	4 (4.8%; 95% CI: 1.9-11.6%)
$\geq 200^1$	71 (68.3%; 95% CI: 58.8-76.4%)	6 (7.1%; 95% CI: 3.3-14.7%)

¹Counts ≥ 200 CFU/100 mL were recorded as TNTC. CI = Confidence interval; TNTC = Too numerous to count.

Antimicrobial susceptibility of surface water isolates

A total of 93 *E. coli* isolates from surface water were included in the antimicrobial susceptibility analysis after exclusion of 11 isolates with duplicate phenotypic profiles. The highest nonsusceptibility was observed against

ampicillin (54.8%; 95% CI: 44.7-64.6%), followed by amoxicillin-clavulanate (19.4%; 95% CI: 12.6-28.5%), gentamicin (18.3%; 95% CI: 11.7-27.3%), tetracycline (15.1%; 95% CI: 9.2-23.5%), and ciprofloxacin (14.0%; 95% CI: 8.3-22.4%). Nonsusceptibility to each of the third-generation cephalosporins CTX and CAZ was 8.6% (95% CI: 4.4-16.1%). All isolates were susceptible to ETP.

Overall, 64.5% (60/93; 95% CI: 54.4-73.5%) of isolates were nonsusceptible to at least one antimicrobial agent, and 19.4% (18/93; 95% CI: 12.6-28.5%) were MDR. ESBL production was detected in 5.4% (5/93; 95% CI: 2.3-12.0%) of isolates originating from five distinct sites, whereas no presumptive AmpC-producing isolates were identified. The overall resistance profile is presented in Figure 3.

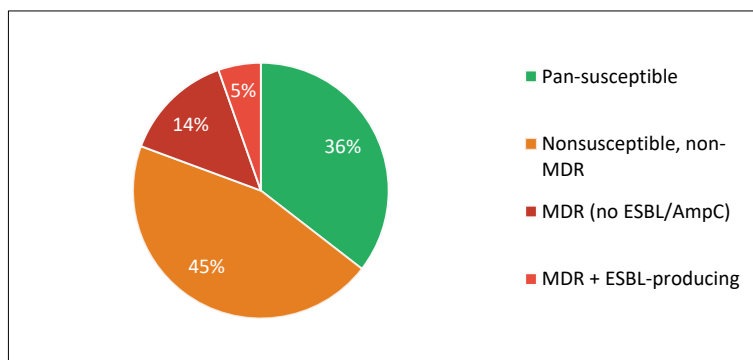


Figure 3: Antimicrobial susceptibility profiles of *Escherichia coli* isolates from surface water sources in Gampaha District. Percentages may not sum to 100% due to rounding.

Antimicrobial susceptibility of groundwater isolates

Among the 84 groundwater *E. coli* isolates, the highest nonsusceptibility was observed against ampicillin (45.2%; 95% CI: 35.0-55.9%), followed by tetracycline and amoxicillin-clavulanate (17.9% each; 95% CI: 11.1-27.4%), ciprofloxacin (16.7%; 95% CI: 10.2-26.0%), and gentamicin (13.1%; 95% CI: 7.6-21.6%). Nonsusceptibility to both CTX and CAZ was 7.1%. All isolates were susceptible to ETP. ESBL production was detected in 1.2% (1/84) of isolates, whereas presumptive AmpC production was detected in 2.4% (2/84). The overall resistance profile is presented in Figure 4.

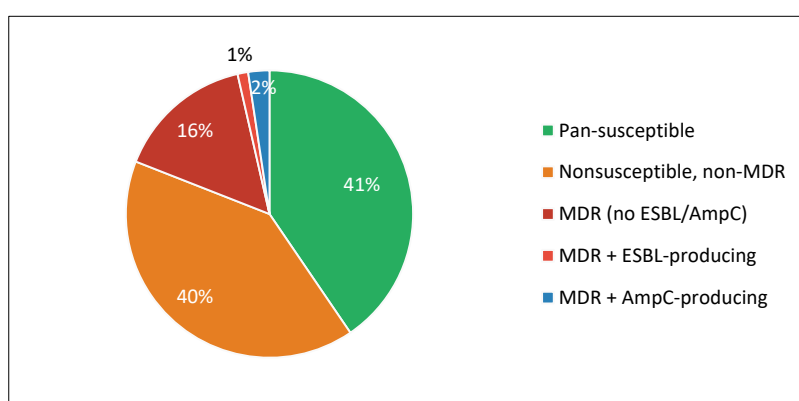


Figure 4: Antimicrobial susceptibility profiles of *Escherichia coli* isolates from groundwater sources in Gampaha District. Percentages may not sum to 100% due to rounding.

Comparison of AMR patterns between surface water and groundwater isolates

Nonsusceptibility rates were generally similar between surface water and groundwater isolates (Table 4). Surface water isolates had numerically higher nonsusceptibility to ampicillin (54.8% vs. 45.2%) and gentamicin (18.3% vs. 13.1%); however, neither difference was statistically significant.

The distribution of nonsusceptibility categories—susceptible to all classes, nonsusceptible to one class, nonsusceptible to two classes, and MDR—did not differ significantly between surface water and groundwater isolates ($\chi^2 = 2.277$, $df = 3$, $p = 0.517$; Table 5). The number of nonsusceptible antimicrobial classes also did not differ significantly among water body types (Kruskal-Wallis $H = 1.690$, $p = 0.639$), and MDR prevalence did not differ according to water body type ($\chi^2 = 0.754$, $df = 3$, $p = 0.860$). Despite the substantial difference in contamination intensity between surface water and groundwater, MDR prevalence was similar in the two source types (19.4% and 19.0%, respectively).

Table 4: Antimicrobial nonsusceptibility rates of *Escherichia coli* isolates from surface water and groundwater sources in Gampaha District

Antimicrobial agent	Surface water (n = 93), n (%; 95% CI)	Groundwater (n = 84), n (%; 95% CI)	p-value
Ampicillin	51 (54.8%; 44.7-64.6%)	38 (45.2%; 35.0-55.9%)	0.230
Amoxicillin-clavulanate	18 (19.4%; 12.6-28.5%)	15 (17.9%; 11.1-27.4%)	0.848
Gentamicin	17 (18.3%; 11.7-27.3%)	11 (13.1%; 7.5-21.9%)	0.412
Tetracycline	14 (15.1%; 9.2-23.7%)	15 (17.9%; 11.1-27.4%)	0.686
Ciprofloxacin	13 (14.0%; 8.4-22.5%)	14 (16.7%; 10.2-26.1%)	0.678
Trimethoprim-sulfamethoxazole	9 (9.7%; 5.2-17.4%)	12 (14.3%; 8.4-23.3%)	0.363
Cefotaxime	8 (8.6%; 4.4-16.1%)	6 (7.1%; 3.3-14.7%)	0.786
Ceftazidime	8 (8.6%; 4.4-16.1%)	6 (7.1%; 3.3-14.7%)	0.786
Chloramphenicol	2 (2.2%; 0.6-7.5%)	2 (2.4%; 0.7-8.3%)	1.000
Ertapenem	0 (0%; 0.0-4.0%)	0 (0%; 0.0-4.4%)	—

CI = Confidence interval. P-values were calculated using Fisher's exact test (two-tailed). Fisher's exact test was used throughout because of small expected cell counts for some antimicrobial agents.

Table 5: Distribution of antimicrobial nonsusceptibility patterns among *Escherichia coli* isolates from surface water and groundwater sources in Gampaha District

Resistance pattern	Surface water (n = 93), n (%; 95% CI)	Groundwater (n = 84), n (%; 95% CI)	p-value†
Susceptible to all classes	33 (35.5%; 26.5-45.6%)	34 (40.5%; 30.6-51.2%)	0.537
Nonsusceptible to 1 class	29 (31.2%; 22.7-41.2%)	28 (33.3%; 24.2-43.9%)	0.872
Nonsusceptible to 2 classes	13 (14.0%; 8.4-22.5%)	6 (7.1%; 3.3-14.7%)	0.155
Nonsusceptible to ≥3 classes (MDR)	18 (19.4%; 12.6-28.5%)	16 (19.0%; 12.1-28.7%)	1.000

Overall comparison: $\chi^2 = 2.277$, df = 3, p = 0.517. CI = Confidence interval; MDR = Multidrug resistance. †Per-row p-values were calculated using Fisher's exact test (two-tailed), with each category compared with all remaining isolates combined; all comparisons were non-significant (p > 0.05).

Binary logistic regression identified no statistically significant predictors of MDR. Neither water body type (adjusted odds ratio [aOR] = 0.63; 95% CI: 0.21-1.95; p = 0.424) nor log-transformed *E. coli* count (aOR = 1.65; 95% CI: 0.41-6.64; p = 0.484) was significantly associated with MDR (likelihood-ratio $\chi^2 = 0.919$, p = 0.632). The correlation between colony-count and the number of nonsusceptible antimicrobial classes was weak and non-significant in both surface water (p = 0.142, p = 0.174) and groundwater (p = 0.099, p = 0.369). Similarly, point-biserial correlations between colony-count and MDR status were non-significant in surface water (r = 0.047, p = 0.656) and groundwater (r = 0.098, p = 0.374). Thus, no statistically significant association between contamination level and MDR status was detected in either source type.

Ten simultaneous antimicrobial comparisons were performed without adjustment for multiple comparisons. At $\alpha = 0.05$, the probability of at least one false-positive result across 10 comparisons is approximately 40%; therefore, borderline p-values should be interpreted as exploratory.

Spatial distribution of surface water contamination and AMR

The spatial distribution of *E. coli* contamination and MDR across all 52 surface water sampling sites is presented in Figure 5. Sites with *E. coli* counts ≥ 200 CFU/100 mL and sites yielding MDR isolates were distributed across divisional secretariat areas of the district, including rural areas. ESBL-producing isolates were detected at five geographically dispersed sites.

Figure 5A shows the spatial distribution of *E. coli* contamination by colony-count category, with ESBL-positive sites marked by star symbols. Figure 5B shows the distribution of MDR and non-MDR isolates across the 52 sites. Contamination burden did not differ significantly among urban, peri-urban, and rural site classifications (Kruskal-Wallis H = 3.582, p = 0.167). The five sites yielding ESBL-producing isolates occurred in both urban/peri-urban and rural areas.

Summary comparison of surface water and groundwater

A summary of the principal contamination and nonsusceptibility parameters for surface water and groundwater is presented in Table 6. *E. coli* positivity was significantly higher in surface water than in groundwater (100% vs. 52.2%, p < 0.001). The proportion with counts ≥ 200 CFU/100 mL was also higher in surface water than in *E. coli*-positive groundwater samples (68.3% vs. 7.1%, p < 0.001). In contrast, the proportions of isolates nonsusceptible to at least one antimicrobial agent (64.5% vs. 59.5%), MDR isolates (19.4% vs. 19.0%), ESBL-producing isolates (5.4% vs. 1.2%), and presumptive AmpC-producing isolates (0% vs. 2.4%) did not differ significantly between source types (all p > 0.05). All isolates from both sources remained susceptible to ETP.

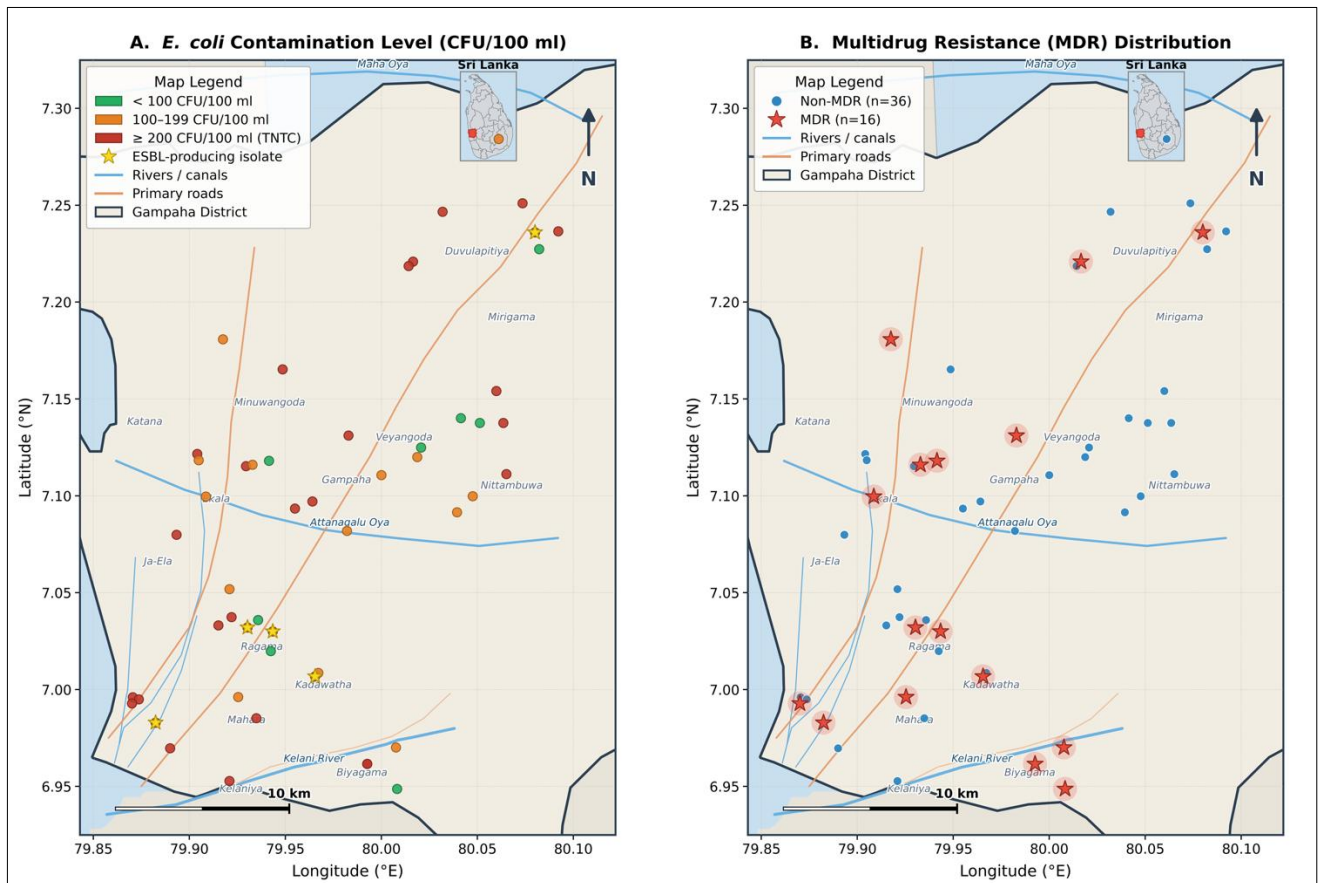


Figure 5: Spatial distribution of *E. coli* contamination levels and MDR among surface water sampling sites in Gampaha District (n = 52). (A) Spatial distribution of *E. coli* contamination according to colony-count: green = <100 CFU/100 mL; orange = 100–199 CFU/100 mL; red = ≥200 CFU/100 mL (TNTC); yellow star = Site yielding an ESBL-producing isolate. (B) Spatial distribution of MDR: blue circle = Non-MDR; red star = MDR. ESBL = Extended-spectrum beta-lactamase; MDR = Multidrug resistance; TNTC = Too numerous to count.

Table 6: Summary comparison of microbiological and antimicrobial susceptibility parameters between surface water and groundwater *Escherichia coli* isolates in Gampaha District.

Parameter	Surface water (93 isolates; 104 samples)	Groundwater (84 isolates; 161 samples)	Difference‡	p-value
<i>E. coli</i> positivity	100% (104/104)	52.2% (84/161; 95% CI: 44.5–59.7%)	+47.8 pp	<0.001
TNTC (≥200 CFU/100 mL)	68.3% (71/104; 95% CI: 58.8–76.4%)	7.1% of positive samples (6/84; 95% CI: 3.3–14.7%)	+61.2 pp	<0.001
Nonsusceptible to ≥1 antimicrobial agent	64.5% (60/93; 95% CI: 54.4–73.5%)	59.5% (50/84; 95% CI: 48.8–69.4%)	+5.0 pp	0.537
MDR	19.4% (18/93; 95% CI: 12.6–28.5%)	19.0% (16/84; 95% CI: 12.1–28.7%)	+0.4 pp	0.517†
ESBL-producing isolates	5.4% (5/93; 95% CI: 2.3–12.0%)	1.2% (1/84; 95% CI: 0.2–6.4%)	+4.2 pp	0.214
Presumptive AmpC-producing isolates	0% (0/93)	2.4% (2/84; 95% CI: 0.7–8.3%)	–2.4 pp	0.224
Highest resistance	Ampicillin: 54.8% (95% CI: 44.7–64.6%)	Ampicillin: 45.2% (95% CI: 35.0–55.9%)	+9.6 pp	0.230
ETP resistance	0%; all susceptible	0%; all susceptible	—	—

CI = Confidence interval; ESBL = Extended-spectrum beta-lactamase; MDR = Multidrug resistance; pp = Percentage points; TNTC = Too numerous to count. p values were calculated using Fisher's exact test (two-tailed), unless otherwise indicated. †The MDR p-value corresponds to the overall Chi-square comparison of resistance-category distributions ($\chi^2 = 2.277$, df = 3). ‡Difference = Surface water minus groundwater; positive values indicate higher proportions in surface water.

DISCUSSION

This study provides district-wide comparative evidence on environmental AMR in surface water and groundwater in Gampaha District, Sri Lanka. Three features strengthen its contribution to environmental AMR surveillance. First, surface water and groundwater were evaluated simultaneously within the same administrative district, allowing direct comparison of contamination and resistance patterns between water-source types. Second, GPS-based spatial mapping was integrated with standardized documentation of human exposure activities at 52 surface water bodies. Third, the findings were considered within a One Health framework and in relation to Sri Lanka's National Strategic Plan for Combating AMR 2023–2028, thereby providing a basis for locally targeted surveillance and intervention priorities. Together, these elements extend previous Sri Lankan

investigations that focused on individual water bodies, selected environmental settings, or nationally aggregated data.

Contamination levels and environmental drivers

All 52 surface water sites were positive for *E. coli*, and 68.3% of surface water samples contained >200 CFU/100 mL. These contamination levels exceeded the thresholds referenced in the Sri Lankan SLS 614:2013 standard [30] and World Health Organization recreational water guidance [31]. In comparison, 52.2% of groundwater samples were positive for *E. coli*, which is broadly consistent with previous Sri Lankan studies reporting contamination in 57-90% of sampled wells [32, 33]. These findings indicate substantial fecal contamination across both environmental water-source types, although the contamination burden was markedly greater in surface water.

Several environmental and anthropogenic factors may contribute to the observed contamination patterns. Gampaha District combines high population density, rapid peri-urban development, intensive agricultural activity, industrial development, and widespread dependence on decentralized sanitation systems. Inadequate sewage treatment, leakage from septic systems, and domestic, agricultural, and industrial effluent discharge may therefore contribute to microbial contamination of local water systems [34]. The presence of the Biyagama Export Processing Zone, hospitals, pharmaceutical outlets, paddy cultivation, and aquaculture within a shared hydrological landscape further increases the potential for multiple contamination sources. However, because specific pollutant sources and land-use exposures were not quantified in the present study, their individual contributions to the observed contamination cannot be determined.

Patterns and potential drivers of AMR

Ampicillin resistance was the most common resistance phenotype in both surface water (54.8%) and groundwater (45.2%) isolates. This finding is consistent with the high national consumption of penicillins in Sri Lanka, where this class accounts for approximately 60% of antibiotic use [12]. High antimicrobial consumption may increase selection pressure for resistant bacteria that subsequently enter aquatic environments through wastewater, sewage, and other fecal contamination pathways. The present findings are also consistent with the high prevalence of amoxicillin resistance previously reported among *E. coli* isolates from inland waters in Sri Lanka [22].

Antibiotic residues, including amoxicillin and penicillin, have previously been detected in the Kelani River [20]. Such environmental residues may contribute to selection and persistence of resistant organisms when present at biologically relevant concentrations. In addition, hospitals, pharmaceutical facilities, agricultural production systems, poultry farms, and aquaculture operations may represent potential sources of antimicrobial residues and resistant bacteria entering surface waters through wastewater and runoff. Studies from South Asia have associated proximity to healthcare facilities and pharmaceutical manufacturing with increased environmental resistance to fluoroquinolones and cephalosporins [35, 36]. Agricultural application of animal manure may also facilitate dissemination of resistant bacteria and ARGs through runoff [14, 37]. Nevertheless, the present study did not measure antibiotic concentrations or source-specific inputs; therefore, these pathways should be considered plausible explanations rather than demonstrated causal mechanisms.

Approximately 19% of isolates from both surface water and groundwater were MDR, indicating the co-occurrence of resistance to multiple antimicrobial classes within a substantial proportion of environmental *E. coli*. Nonsusceptibility to both ampicillin and amoxicillin-clavulanate occurred in 18 surface water and 15 groundwater isolates. Several mechanisms could contribute to this phenotype, including increased beta-lactamase expression or inhibitor-resistant beta-lactamases; however, phenotypic susceptibility testing alone cannot distinguish these mechanisms. Similarly, the co-occurrence of nonsusceptibility to fluoroquinolones and aminoglycosides among some MDR isolates may reflect mobile resistance determinants, but this study did not investigate plasmids, integrons, or specific resistance genes.

Aquatic biofilms are recognized environments in which bacterial persistence and horizontal gene transfer can occur [38], and subinhibitory concentrations of antibiotics may promote the transfer or maintenance of resistance determinants [39]. ESBL-producing *E. coli* were detected in 5.4% of surface water isolates and 1.2% of groundwater isolates. ESBL determinants, particularly *bla*_{CTX-M}-family genes, are frequently plasmid-mediated in Gram-negative bacteria in South Asian settings. However, the present study did not perform genotypic confirmation; therefore, the specific resistance genes, plasmid types, and mechanisms responsible for the observed phenotypes remain unknown. Molecular characterization is needed to determine whether environ-

mental isolates share resistance determinants or phylogenetic relationships with clinical strains circulating in Gampaha District.

Comparison with regional and international data

The MDR prevalence of approximately 19% observed in both water-source types was higher than the 11% reported in Dutch surface water [40], lower than the 75%-85% reported in studies from India and Nepal [41, 42], and broadly comparable to the 15% reported across multiple water-source types in Bangladesh [43]. These comparisons suggest that environmental MDR prevalence varies considerably among geographic settings, likely reflecting differences in antimicrobial use, sanitation infrastructure, wastewater treatment, sampling strategies, bacterial populations, and analytical methods.

Similarly, the prevalence of ESBL-producing *E. coli* in the present study, 5.4% in surface water and 1.2% in groundwater, was substantially lower than the 58% reported in Pakistan and falls within the broad range of 3-64% reported from China [44]. Direct comparison should nevertheless be interpreted cautiously because differences in sampling design, water-source characteristics, isolate selection, and phenotypic or molecular detection methods may substantially influence reported prevalence.

No resistance to ETP was detected among isolates from either source type. This finding differs from reports of carbapenem resistance determinants in Indian river environments [45]. Although the absence of phenotypic ETP resistance is encouraging, continued environmental surveillance remains important because aquatic environments may facilitate the introduction and dissemination of clinically important resistance determinants.

Surface water-groundwater interconnectivity

Despite substantially greater *E. coli* contamination in surface water, AMR-category distributions did not differ significantly between surface water and groundwater isolates ($\chi^2 = 2.277$, $df = 3$, $p = 0.517$), and MDR prevalence was nearly identical (19.4% and 19.0%, respectively). One possible explanation is hydrological connectivity between surface water and shallow groundwater systems in low-lying areas of the district. Surface water infiltration, septic-system leakage, and flood-associated movement of contaminated water may provide pathways for resistant bacteria or resistance determinants to move between environmental compartments. Similar surface water-groundwater connectivity has been described in other South Asian urban settings [43, 46].

The ESBL prevalence was numerically higher in surface water than in groundwater (5.4% vs. 1.2%), whereas presumptive AmpC-producing isolates were detected only in groundwater (2.4%). However, these differences were not statistically significant, and the numbers of ESBL- and presumptive AmpC-producing isolates were small. Consequently, the observed distributions should not be interpreted as evidence of distinct resistance-selection pressures between surface and groundwater without further molecular and environmental investigation.

Seasonal variation and climate-related considerations

Surface water sampling was conducted between November 2023 and February 2024, primarily during the transition from the second inter-monsoon period to the dry season. Consequently, the study does not characterize AMR patterns during peak Southwest monsoon conditions. Increased rainfall and flooding can enhance runoff, mobilize fecal material and antimicrobial residues, and increase hydrological connectivity between contamination sources and receiving water systems [47–49]. As a result, contamination and resistance patterns may differ during wetter periods.

Climate-associated changes may further influence environmental AMR dynamics. Increased water temperatures can affect bacterial growth and horizontal gene transfer, while extreme rainfall can increase the transport of resistant bacteria and antimicrobial residues into aquatic environments [47]. Flooding events have also been associated with increased dissemination of resistant waterborne organisms in South Asia [48, 49]. Furthermore, rising temperatures have been associated with higher AMR prevalence in *E. coli* in broader epidemiological analyses [50]. These observations support the need for longitudinal surveillance across wet and dry seasons, rather than implying that the present estimates necessarily represent the minimum annual AMR burden.

One Health implications

National surveillance data from Sri Lanka have reported ampicillin resistance >80%, ciprofloxacin resistance of 57.7%, trimethoprim-sulfamethoxazole resistance of approximately 60%, and third-generation cephalosporin resistance of 57-61.9% among clinical *E. coli* bloodstream isolates [51, 52]. The corresponding resistance proportions among environmental isolates in the present study were substantially lower: approximately 45-55%

for ampicillin, 14-17% for ciprofloxacin, 10-14% for trimethoprim-sulfamethoxazole, and 7-9% for third-generation cephalosporins.

The presence of resistance to the same antimicrobial classes in clinical and environmental *E. coli* is compatible with circulation of related resistance determinants across human and environmental compartments, although the present study did not establish direct transmission or genomic relatedness. A national One Health systematic review reported median ampicillin resistance of 60% and sulfamethoxazole resistance of 88.8% across human, animal, and environmental *E. coli* isolates [53], further demonstrating the widespread occurrence of AMR across sectors in Sri Lanka.

Several pathways could connect these compartments, including discharge of hospital and community wastewater, runoff from poultry and aquaculture production, manure application to agricultural land, and human exposure to contaminated water during bathing, fishing, irrigation, and other activities. These findings therefore support including environmental monitoring in integrated One Health AMR surveillance. However, genomic comparison of human, animal, and environmental isolates is needed to demonstrate transmission pathways and determine whether resistance determinants are shared among sectors.

Implications for surveillance and public health action

The findings identify several priorities for strengthening environmental AMR surveillance and water safety interventions. At the national level, priorities include improving wastewater and sewage treatment infrastructure, strengthening antimicrobial stewardship across human and veterinary medicine and food-production systems, expanding community water safety education, and formally incorporating environmental AMR surveillance into implementation of the National Strategic Plan for Combating AMR 2023-2028 [54].

At the district level, surveillance and risk communication could prioritize surface water sites with high microbial contamination and documented human contact. The Dutch Canal and Hamilton Canal in Wattala, the Kelani River in Kelaniya, the Ja Ela River, and Peliyagoda Lake were identified as heavily contaminated locations where human exposure was documented. Appropriate responses could include enhanced microbiological monitoring, public risk communication, evaluation of safe-water alternatives, and investigation of potential upstream contamination sources. Sites yielding ESBL-producing isolates near healthcare or industrial areas may also warrant targeted environmental investigation and assessment of wastewater-management practices.

For groundwater, expansion of PHI-led monitoring could improve characterization of AMR in drinking water wells, particularly if future surveillance incorporates systematic sampling, well type, depth, construction characteristics, geographic location, and potential contamination sources. Coordination among public health, environmental, water-management, and agricultural authorities would strengthen the One Health value of such surveillance.

Quantifying health risk through quantitative microbial risk assessment (QMRA)

The present study characterized contamination, exposure activities, and AMR phenotypes but did not directly quantify the probability of adverse human health outcomes. QMRA provides a framework for estimating infection risk associated with defined waterborne exposures [55] and has been identified as an important approach for water safety assessment in low- and middle-income settings [56].

The exposure pathways documented in this study, including bathing, fishing, irrigation, drinking water use, wastewater inputs, agricultural runoff, and potential groundwater infiltration, could provide a basis for future QMRA scenarios. Previous QMRA studies evaluating ESBL-producing *E. coli* in recreational water have indicated that some exposure scenarios may exceed accepted health-risk thresholds [57]. Incorporating site-specific exposure frequency, ingestion volumes, pathogen concentrations, and dose-response relationships would allow future studies to translate environmental contamination data into quantitative estimates of human health risk.

QMRA could also help compare potential interventions, such as wastewater treatment improvements, well protection, restrictions on high-risk water contact, and provision of alternative water sources. Such analyses may provide policymakers with a more quantitative basis for prioritizing investments in environmental AMR surveillance and mitigation.

Limitations and future research directions

This study has several limitations. First, surface water sampling was restricted to November 2023-February 2024 and therefore did not capture peak Southwest monsoon conditions. Seasonal changes in rainfall, runoff, water flow, and fecal contamination may influence both bacterial abundance and AMR patterns. Future studies should incorporate repeated sampling during wet and dry seasons to characterize temporal variability.

Second, ESBL and AmpC phenotypes were not confirmed genotypically. Phenotypic methods cannot identify specific resistance determinants such as *bla*_{CTX-M} or *bla*_{CMY} genes, characterize plasmid-mediated transmission, or establish phylogenetic relationships with clinical isolates. Future studies should therefore incorporate polymerase chain reaction-based resistance-gene characterization and whole-genome sequencing to define the environmental resistome and investigate relationships among environmental, human, and animal isolates.

Third, groundwater samples originated from routine PHI-led surveillance rather than probability-based sampling. Wells suspected of contamination or serving particular populations may therefore have been preferentially submitted, whereas remote or less frequently monitored wells may have been underrepresented. Consequently, groundwater prevalence estimates should be interpreted as representative of the monitored well population rather than the entire groundwater supply of Gampaha District. Information on well type, depth, casing, and structural condition was also unavailable, preventing assessment of these factors as potential predictors of contamination.

Fourth, the study did not measure antimicrobial residue concentrations. The study therefore cannot distinguish between resistance selected *in situ* within aquatic environments and resistant organisms introduced from human, animal, agricultural, or wastewater sources. Future studies integrating microbiological, genomic, and chemical measurements would provide stronger evidence regarding environmental selection pressures.

Fifth, culture-based methods may not detect viable but nonculturable *E. coli* [58]. In addition, other relevant fecal indicator or antimicrobial-resistant organisms, including *Enterococcus* spp., were not examined [59]. Broader culture-independent and metagenomic approaches could provide a more comprehensive assessment of environmental AMR.

Sixth, the number of ESBL-producing isolates was small ($n = 5$ in surface water and $n = 1$ in groundwater), resulting in imprecise prevalence estimates. No correction for multiple comparisons was applied across the 10 antimicrobial agents; therefore, borderline p-values should be interpreted as exploratory. The logistic regression model was also restricted to two covariates because potentially relevant variables, including land-use, proximity to hospitals or industrial facilities, wastewater discharge, and other contamination sources, were not systematically recorded.

Finally, the study was restricted to Gampaha District. Extrapolating the findings to other regions of Sri Lanka or South Asia should therefore be done cautiously because environmental conditions, sanitation infrastructure, antimicrobial use, land use, and hydrological characteristics may differ substantially across settings. Nevertheless, the surveillance framework used here provides a basis for comparable district level investigations elsewhere in Sri Lanka.

CONCLUSION

This district-wide study demonstrated substantial *E. coli* contamination and clinically relevant AMR in both surface water and groundwater in Gampaha District, Sri Lanka. All 52 surface water sites were positive for *E. coli*, and 68.3% of surface water samples contained ≥ 200 CFU/100 mL, whereas 52.2% of groundwater samples were *E. coli*-positive. Among isolates subjected to AST, 64.5% of surface water isolates and 59.5% of groundwater isolates showed nonsusceptibility to at least one antimicrobial agent. MDR prevalence was similar in the two source types (19.4% and 19.0%, respectively), while ampicillin showed the highest nonsusceptibility in both surface water (54.8%) and groundwater (45.2%) isolates. ESBL-producing *E. coli* were detected in both source types, whereas presumptive AmpC-producing isolates were identified only in groundwater. All isolates remained susceptible to ertapenem.

A major strength of this study was the simultaneous district-wide evaluation of surface water and groundwater within the same administrative setting, combined with GPS-based spatial mapping and standardized documentation of human exposure activities at 52 surface water bodies. This integrated design enabled direct comparison of contamination and AMR profiles across water-source types and provided a high-resolution environmental AMR baseline for Gampaha District. The comparable MDR burden observed despite markedly different levels of *E. coli* contamination further emphasizes the importance of evaluating AMR independently of contamination intensity alone.

Overall, the findings show that environmental AMR is widely distributed across both surface water and groundwater systems in Gampaha District and support including environmental monitoring in integrated One Health AMR surveillance. The study also provides a practical framework for district level surveillance that can inform targeted water safety interventions and future monitoring strategies. Continued surveillance should

incorporate repeated wet- and dry-season sampling, molecular characterization of resistance determinants, broader environmental exposure assessment, and quantitative microbial risk assessment to strengthen understanding of transmission pathways and human health implications. These findings provide an evidence base for coordinated local and national actions aimed at limiting the environmental dissemination of antimicrobial-resistant bacteria in Sri Lanka.

DATA AVAILABILITY

The datasets generated and analyzed during the current study are available in the Science Data Bank repository: Science Data Bank dataset (https://www.scidb.cn/en/s/m6BjQb?utm_source=chatgpt.com). The deposited spatial dataset and isolate data provide a resource for future longitudinal surveillance and genomic investigations.

GENERATIVE AI DECLARATION

During the preparation of this manuscript, the authors used generative artificial intelligence (AI) tools solely to improve the language, grammar, readability of the text and create Figure 5. These tools were not used to generate scientific content, analyse data, interpret findings, or draw conclusions. All outputs were reviewed and edited by the authors, who take full responsibility for the accuracy, integrity, and originality of the published work. No AI tool meets the criteria for authorship, and none has been listed as an author.

AUTHORS' CONTRIBUTIONS

SJ: Conceptualization, investigation, data collection, formal analysis, and writing – original draft. SJ, NB, and SP: Methodology. NB and SP: Resources and supervision. NB: Writing – review and editing. All authors have read and approved the final version of the manuscript.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

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